

# Communicating Research to the General Public

---

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 20 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of \$250 for UW-Madison chemistry Ph.D. candidates.



The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

**UW-Madison Department of Chemistry**  
**1101 University Avenue**  
**Madison, WI 53706-1396**  
**Contact: Prof. Bassam Z. Shakhashiri**  
**bassam@chem.wisc.edu**  
**www.scifun.org**

May 2014

**SPECTROSCOPIC AND COMPUTATIONAL INVESTIGATION  
OF ADENOSYLCOBALAMIN-DEPENDENT ENZYMES  
AND A MEMBRANE-BOUND FATTY ACID DESATURASE**

By

Christopher D. Jordan

A dissertation submitted in partial fulfillment of  
the requirements for the degree of

Doctor of Philosophy

(Chemistry)

at the

UNIVERSITY OF WISCONSIN-MADISON

2015

Date of final oral examination: July 21, 2015

This dissertation is approved by the following members of the Final Oral Committee:

Thomas C. Brunold, Professor of Chemistry - Inorganic  
Qiang Cui, Professor of Chemistry - Physical  
Martin T. Zanni, Professor of Chemistry - Physical  
Silvia Cavagnero, Professor of Chemistry - Physical  
Brian G. Fox, Professor of Biochemistry

## **CHAPTER 8**

*How to Break a Bond:*

*A Brief, Non-Technical Account of Coenzyme B<sub>12</sub>, One Enzyme that Uses It, and the Last Six Years of My Life*

## 8.1 Introduction and Motivation

Over the past six years, I've had the privilege of devoting much of my working life to studying one of nature's most fascinating molecules, coenzyme B<sub>12</sub>. Over those same six years, I have been surrounded by an incredibly supportive network of friends and family who have frequently, out of curiosity or politeness, asked about my research. The answers they have received have often been rambling, circuitous, and disjointed: it turns out that distilling the essence of years of research into a few talking points over dinner or a cup of coffee is challenging. But despite this barrier, I am still excited at the thought of sharing the work I do with these supporters and with the general public. For that reason, I am including this brief, non-technical explanation of my favorite part of the following thesis (Chapter 3). My hope is that this short chapter will be able to rectify some of my past struggles to communicate my research and its significance to those people who kept me sane throughout the process.

Of course, a major challenge in communicating any scientific work to a non-scientific audience is the matter of translating precise technical jargon into words better suited for general communication. In chemistry, however, there are many concepts for which the technical term is the **only** term I can use. To keep this from posing too large of a problem, we'll begin this chapter with two short reviews for non-chemists. The first will help readers understand what exactly coenzyme B<sub>12</sub> is, its biological role, and why chemists are fascinated by it. The second will explain the experimental and theoretical tools we use to investigate it. Following those sections, I will present some of my data and explain how these results have improved the scientific community's understanding of B<sub>12</sub>.

## 8.2 From Atoms to Enzymes: Bonding, Biochemistry, and Catalysis

When I teach freshman chemistry students, I like to begin the first day of class by asking them to take 30 seconds to write down a definition of the word *chemistry*. I don't do this to be cruel to the students, but because it can be instructive to realize how broad a field chemistry is. Any one-sentence definition of chemistry is unlikely to include the work of everyone who self-identifies as a chemist. With that caveat in place, I'll suggest that most chemists are mainly interested in finding out one of two things: (1) why some type of "stuff" has the properties it has, or (2) how to convert one type of "stuff" into another type of "stuff" via what is called a chemical reaction.<sup>1</sup> Even fairly simple chemical questions fall into these two categories. For example, a budding chemist interested in the properties of stuff may try to answer the questions, "Why don't oil and water mix?" or "Why do metals conduct electricity?" Another chemist interested in changing one type of stuff to another might ask, "How does my body use food and oxygen to provide me with energy?"

If chemistry encompasses any question that fits into either of these two categories, then it is an absolutely enormous discipline. Everything we do requires stuff, and everything we see is made of stuff. Stuff makes up our computers, our cars, our food, our environment, our clothes, and, most importantly, ourselves. What ties the study of all of these things together is an audacious two-part claim. First, chemists think that all stuff is made of very small components called atoms. Second, chemists believe that if we understand how atoms are constructed and interact with each other, then we can explain and predict the properties and behaviors of all kinds of stuff. In the remainder of this section, we'll briefly discuss the components of atoms, how atoms come together

---

<sup>1</sup> If you want to sound more scientific, replace "stuff" with "matter" in the previous sentence. I will continue using "stuff."

to form molecules, the special types of molecules involved in my research, and the chemical reactions they perform.

**8.2.1. Atoms are made of protons, neutrons, and electrons.** Atoms consist of some number and arrangement of three **sub-atomic particles** called protons, neutrons, and electrons. These particles differ in three significant ways. First, in the electrical charge they carry: protons have a positive charge, electrons have a negative charge, and neutrons are, well, neutral. Overall, the atoms themselves are also neutral, since they have an equal number of protons and electrons, causing their charges to cancel out. Sub-atomic particles also differ in mass: protons and neutrons have similar weights (neutrons are just a pinch heavier), but both are about 2000 times heavier than electrons. Third, they are found in different places inside the atom. All of the protons and neutrons are very densely packed in a nucleus at the center of the atom, while the tiny electrons swarm about the nucleus at some distance. The distance at which the furthest electrons are found from the nucleus tells us the size of the atom, which is typically about 0.000 000 000 200 meters, or 200 “picometers.” The nucleus itself is about 100,000 times smaller: 0.000 000 000 000 000 002 meters, or 0.002 picometers.

That’s more than just a nice trivia fact. It’s an important observation because it tells us that an atom’s electrons are what communicate with the rest of the world – the nucleus is too small and tucked away to be of primary importance. So if we want to understand how atoms come together to form different types of materials, we need to understand where the electrons are inside of atoms, and how electrons from one atom can interact with electrons from other atoms. This is a very challenging problem to solve, since electrons can behave in very counterintuitive ways. Thankfully, chemists have discovered useful patterns that help us predict how atoms behave based on how many electrons they have. To show the importance of the number of electrons in each

atom, consider this: if you gather a bunch of atoms that each have six electrons and six protons, you'll find that you have carbon (C) in one of its various forms (e.g., graphite, charcoal, diamond). Or, collect a bunch of atoms with seven electrons and you'll have nitrogen (N); choose eight, and you'll get oxygen (O). These materials – carbon, nitrogen, oxygen, and anything else you find on chemistry's periodic table – are examples of **elements**, or materials consisting of only one type of atom. There are about 115 known elements, many of which do not occur naturally. Each element contains only atoms with a characteristic number of electrons and protons.

**8.2.2. Atoms can combine to make molecules by sharing electrons.** There are way more than 115 different types of stuff in the universe, however, so we're going to need to understand materials other than elements. These other materials are called **compounds**, and they contain atoms of more than one kind of element. A common example is water. Many people know its chemical **formula**,  $\text{H}_2\text{O}$ , which describes the ratio of hydrogen to oxygen atoms (2:1) in any sample of water. Water is an example of a **molecular compound**, which means that there are very small distinct units of water called **molecules**, each of which has two hydrogen atoms and one oxygen atom. All of the compounds that I study are also molecular compounds, but they have many more atoms than water. For example, coenzyme B<sub>12</sub> has the formula  $\text{C}_{72}\text{H}_{100}\text{CoN}_{18}\text{O}_{17}\text{P}$ , and ethanolamine ammonia lyase (EAL), another important molecule in this chapter, has the formula  $\text{C}_{3415}\text{H}_{5158}\text{N}_{905}\text{O}_{887}\text{S}_{26}$ .<sup>2</sup>

A major focus of my research is understanding how the many atoms in my molecules are held together. We call the connection between two atoms a **chemical bond**, and bonds within molecules are typically formed when two atoms share a pair of electrons. These electrons benefit

---

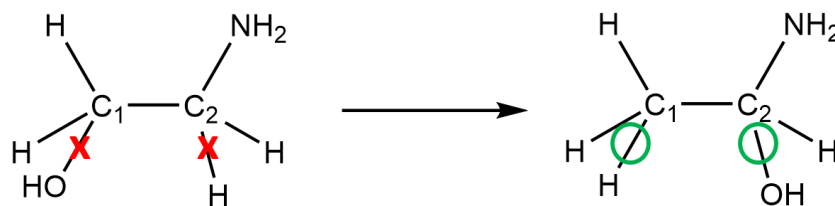
<sup>2</sup> In these formulas, Co stands for cobalt, P stands for phosphorous, and S stands for sulfur.

from the arrangement because as negatively charged particles they are attracted to the positively charged nuclei of both atoms. By spending some time near both nuclei, they force the nuclei to stay close together, and in doing so form a bond between the two atoms. The number of electrons in individual atoms determines how many bonds they will form, how strong those bonds are, and how far apart the bonded atoms sit. Carbon, for example, has atoms that like to form a lot of strong bonds, a property that makes it the centerpiece of a huge class of stable molecules that make up the basis of all living things. On the other hand, helium atoms completely refuse to form bonds at all.

When molecules engage in a **chemical reaction**, bonds may be broken and new bonds may be formed, changing the connections between atoms. For example, when hydrogen ( $\text{H}_2$ ) and oxygen ( $\text{O}_2$ ) molecules are reacted to form water ( $\text{H}_2\text{O}$ ), the bond between the two hydrogen atoms is broken, as is the bond between the two oxygen atoms. As this happens, new bonds are formed between oxygen and hydrogen atoms – it is these bonds that hold the new water molecule together. Other chemical reactions may be more subtle. This chapter is about a rearrangement reaction, which changes which atoms within a molecule are bound to each other. In Scheme 8.1 below, the molecule on the left is called **ethanolamine**, and it has two carbon atoms, which I've labeled  $\text{C}_1$  and  $\text{C}_2$ . Before the reaction happens, there is an "OH" on  $\text{C}_1$  and an "NH<sub>2</sub>" on  $\text{C}_2$ . After the reaction happens, both the "OH" and the "NH<sub>2</sub>" are on  $\text{C}_2$ . It looks like a simple reaction, but it actually is very challenging for two "groups" on one molecule to switch places on their own. This reaction is one step in a multi-reaction sequence that some bacteria use to take carbon dioxide ( $\text{CO}_2$ ) and use it as a source of carbon for all of the complicated molecules they plan to build. Unfortunately, because the reaction is so difficult, it takes a very long time to occur, which poses a big problem

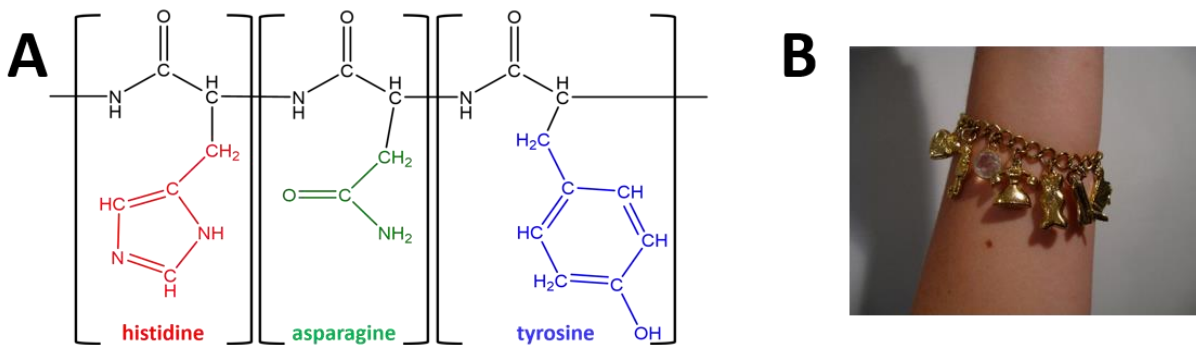


for these bacteria. However, organisms have evolved their own complicated machinery called **proteins** that can **catalyze** reactions. Let's talk about those bolded terms next.



**Scheme 8.1.** The rearrangement of ethanolamine. Lines represent bonds between two atoms. Not all bonds involving hydrogen atoms are drawn. On the left, a red “X” is placed over bonds that are broken during the reaction. Bonds formed during the reaction are circled on the right.

**8.2.3. Proteins: nature’s modular architecture.** In the previous section, I told you that the formula for EAL was  $C_{3415}H_{5158}N_{905}O_{887}S_{26}$ : add up all those subscripts, and you’ll find that this molecule has 10,391 atoms in it. That’s a lot to keep track of, but proteins are constructed in a very regular pattern that helps us understand how they function. Take a look at Figure 8.1A below, where I’ve drawn a small segment of a protein, and note how the sections in black are repeated over and over again. This is the protein’s backbone, a sequence of carbon, nitrogen, and hydrogen atoms that goes on and on and on. The only variation is found in the colored “side chains,” where different groups of atoms containing carbon, hydrogen, nitrogen, oxygen, and sulfur dangle off of the backbone. Living things construct proteins by joining together smaller molecules called **amino acids**: each set of brackets encloses a different amino acid. The three amino acids I included in this figure – histidine, asparagine, and tyrosine – are very important to my research.



**Figure 8.1.** General organization of a protein. (A) Each segment in brackets represents an individual amino acid. Colored groups of atoms are amino acid side chains. Lines extending to the left and right of the figure indicate that more amino acids may be added to either side. (B) A charm bracelet is an analogy for the protein.<sup>3</sup> The charms represent the protein's amino acid side chains, and the links are the backbone that connects the amino acids together.

One way to think of a protein is like a charm bracelet, where each amino acid represents one link in the bracelet's chain (Figure 8.1B). Just as different charms dangle from the jewelry, different amino acids have different groups of atoms that branch off from the protein's backbone. And just like the same individual charms can be reorganized into many different sequences, each different from the other, a vast array of proteins can be built from the same building blocks. In fact, the function of a protein depends on what amino acids are present and what order they are found in. There are twenty different amino acids found commonly in nature, and proteins can be anywhere from a few dozen to thousands of amino acids in length. Although the structure in Figure 8.1A is drawn in roughly a straight line, proteins tend to fold into very complicated three-dimensional structures with lots of nooks and crannies.

Proteins serve a wide variety of functions for healthy organisms. Some of them, like keratin, provide structure for the organism's cells and are responsible for the texture of hair and nails in mammals. Others are responsible for transporting small molecules or nutrients throughout

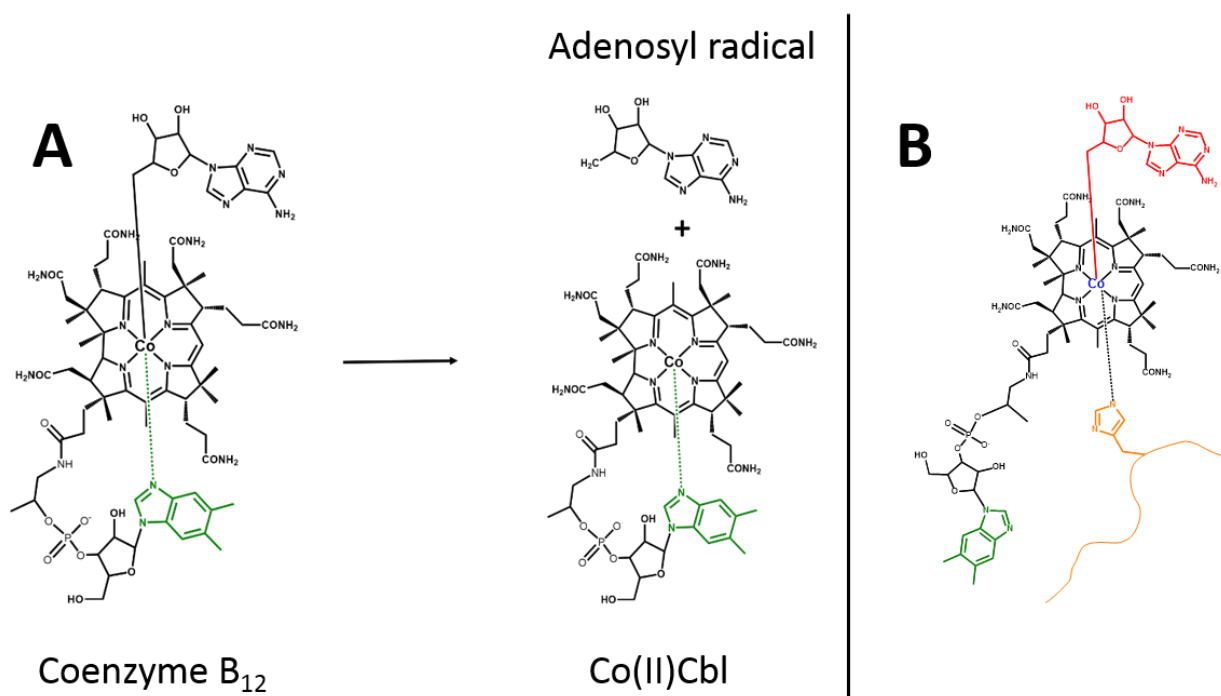
<sup>3</sup> Image credit: Wikipedia.

the organism – right now, a protein called hemoglobin is picking up oxygen from your lungs and will soon deliver it to the rest of your body. But EAL, the protein I work with, is an **enzyme**, which means it is a protein that serves as a **catalyst** for a chemical reaction. Specifically, EAL is the catalyst for the reaction in Scheme 8.1, which means that it helps the reaction go much, much faster – up to 1,000,000,000,000 times faster, to be precise. In technical terms, we say that ethanolamine is EAL's **substrate**. Enzymes and substrates often have a very special relationship in which the substrate fits somewhere into or onto its enzyme, and the enzyme does *something* to make the substrate more reactive.

**8.2.4. EAL, coenzyme B<sub>12</sub>, and ethanolamine.** Despite the crash-course in chemistry you just received, there is one lingering matter that we haven't addressed – the role of coenzyme B<sub>12</sub> (Figure 8.2A). Coenzyme B<sub>12</sub> is a type of molecule called a **cofactor**. While in many ways an enzyme is like a car's engine, chugging away and efficiently converting reactants to products, a cofactor is more like a key in the car's ignition. EAL can't begin to do its work unless coenzyme B<sub>12</sub> is present. For anything to happen in this biochemical machine, then the trio of EAL, coenzyme B<sub>12</sub>, and ethanolamine must all be present. That sentence right there is the inspiration for my research: **Something about the way these three molecules interact with each other allows catalysis to occur, but nobody has a good explanation of what this interaction is.**

It's not at all unusual for enzymes to require cofactors, and EAL is not the only protein that uses coenzyme B<sub>12</sub>, but I care about this particular cofactor for one specific reason. Coenzyme B<sub>12</sub> and a very closely related compound called methylcobalamin possess bonds between a carbon atom and a metal atom (in this case, cobalt, Co), the only known examples of **organometallic**

bonds in all of nature.<sup>4</sup> Moreover, we know that the Co–C bond breaks in the very first step of any reaction for which coenzyme B<sub>12</sub> is required. The breaking of the Co–C bond creates two fragments called an “adenosyl radical” and “cobalt (II) cobalamin,” or “Co(II)Cbl” for short (Figure 8.2A). The former is an extremely reactive molecular fragment that goes on to react with the ethanamine. We know that the rate at which the Co–C bond breaks controls the rate of catalysis for the overall reaction.



**Figure 8.2.** (A) Coenzyme B<sub>12</sub> (left), and the first step of the reactions it helps catalyze. In this structure, hydrogen atoms are omitted for clarity, and bonds between carbon atoms are drawn without the elemental symbol “C”. The DMB group (see text) is colored green. The products of this reaction step are an adenosyl radical (top, right) and Co(II)Cbl (bottom, right). (B) Coenzyme B<sub>12</sub> bound to an enzyme through replacing the DMB (green) with a histidine side chain (orange). The curly line represents the protein backbone, and other amino acids are not shown.

<sup>4</sup> In the interest of full disclosure, several years ago researchers discovered a carbon atom surrounded by six iron atoms in an enzyme called nitrogenase. Coenzyme B<sub>12</sub> is different in that the carbon bound to the cobalt atom is part of a bigger organic “group”, instead of just being a lone atom.

There is one feature of the enzyme-cofactor relationship that is particularly puzzling. First, there are several dozen known enzymes that utilize coenzyme B<sub>12</sub> as a cofactor, and they can be loosely grouped into two classes. One, which contains the only human coenzyme B<sub>12</sub>-dependent enzyme, is known to bind the cofactor by breaking the Co—N bond opposite the Co—C bond, displacing a part of B<sub>12</sub> we call “DMB.”<sup>5</sup> The DMB is replaced by forming a new Co—N bond between the Co and a nitrogen atom from a histidine side chain (Figure 8.2B). For a long time, many scientists thought that this must play a key role in helping enzymes break the Co—C bond, and a lot of work was put into researching how the Co—C bond’s strength depended on the strength of the Co—N bond across from it. Then, the unexpected happened. Researchers discovered that the other class of B<sub>12</sub>-dependent enzymes does not break any Co—N bonds when the cofactor binds. Despite this fact, enzymes in this class are equally effective at weakening the Co—C bond as the others. EAL belongs to this latter class.

Thus, we have a conundrum. Two different classes of enzymes catalyze the same types of reactions, and do so with similar efficiencies. Both classes of enzymes use the same cofactor, and the key step in all reactions using this cofactor is the breaking of its Co—C bond. And yet, one of these classes absolutely requires a histidine side chain to attach to the Co atom to function, and the other has absolutely no need for this. The research presented informally here (and formally in Chapter 3) offers a possible explanation for this.

**8.2.5. Why bother?** Finally, let me address a more important question I’ve been asked many times since beginning this research. **Why?** Why do chemists bother studying things like

---

<sup>5</sup> Short for “**dimethylbenzimidazole**.” I don’t want to type that word out over and over again, and I suspect you don’t want to read it over and over again.

this? The questions I want to answer are very esoteric, and it's taken the better part of six years<sup>6</sup> to gather enough evidence to propose some answers to them. This seems like a very difficult thing to study, and it seems very far removed from other, more practical things we could study, like the nutritional effects of B<sub>12</sub>. In other words, why are we working so hard to understand *how* B<sub>12</sub> works if we already have a good grasp on *what* it can do?

To answer, humor me with this scenario: pretend you've never seen a car before, and that you've stumbled across one in the woods. You find the owner's manual in the glove compartment, and after perusing that you have a general idea of what a car does and how to operate it. Excellent – good for you! But no matter how closely you study the owner's manual, you will never get a real idea of how everything under the hood comes together to make the car work. It would take years of monkeying around, testing out many failed theories of what each component does before you would gain a working knowledge of the car's engine.

Would those years be well-spent? I can think of three possible reasons to answer “Yes,” and each one is directly analogous to a reason for research like my own. First, you might study that car's engine so that you know how to fix it if it breaks down. Similarly, many biochemists and molecular biologists study how coenzyme B<sub>12</sub> functions so that they can think of ways to treat disorders related to its malfunction. For example, some men and women suffer from a genetic disease that affects the human enzyme that uses coenzyme B<sub>12</sub>. When that happens, this particular enzyme becomes unable to bind the cofactor, and the result is a disease called methylmalonic acidemia caused by the toxicity of the unreacted substrate. Currently the best way to treat the disease is a liver transplant. Wouldn't it be nice if, based on our understanding of the interaction

---

<sup>6</sup> To be fair to myself, those other six years did include a lot of coursework, teaching, research related to the other chapters of this thesis, a year away from campus, and – occasionally – a little bit of family time.

between enzymes and coenzyme B<sub>12</sub>, we could design an analogue of the cofactor that was still capable of binding despite this mutation and add that as a supplement to patients' diets? Such lofty goals are unfeasible in the absence of an understanding of how catalysis works.

Second, you might study your mystery car's engine in order to use its technology to accomplish an unrelated purpose. That is, if you want to build a lawnmower, you might take the things you learned from your study of the car engine and adapt them to make them suitable for your new tool. Coenzyme B<sub>12</sub> may be the only known organometallic cofactor, but organometallic catalysts in general have become incredibly useful industrially. To give a sense of this, the American Chemical Society has an entire journal devoted solely to the study of organometallic compounds. Last year alone, nearly 850 articles were published in this journal, and the journal was cited over 42,000 times (cite). Most of the research in this journal is related to the synthesis of new catalysts, and the measurement and explanation of their properties. When it comes to designing these catalysts, however, there are likely some very important lessons that can be taken from coenzyme B<sub>12</sub>. This cofactor, and the enzymes that utilize it, are the products of billions of years of evolutionary pressures that reward those organisms best adapted to their environment and the resources they have available. In other words, the cofactor/enzyme combination is already a well-engineered machine, and its study should bear fruit in fields other than medicine or biochemistry.

Last, many of you might study the car's engine because it's cool. There is something about the human mind that is fascinated by machines. Studying cars or enzymes for this reason isn't pragmatic or utilitarian, but those who lack this inspiration will find it impossible to dedicate years of their lives to study. Nearly every scientist who survives the rigors and setbacks of research is a person who is captivated by a problem, and is willing to devote his or her career to solving it. The scientist in the lab is not all that different from the grease monkey who tinkers for hours late at

night in the garage. Both have a passion that inspires them to a better understanding of their work, which in turn further fuels that passion. For me, B<sub>12</sub> is the most fascinating puzzle I've been exposed to (and subjected to) in my life. Without its inspiration, I would never have spent the countless hours in the lab and in front of the computer necessary to do this research, and I would never have bothered writing this summary for you. Since we're on the subject, let's move on and talk about exactly how these hours were invested.

### 8.3 Matter, Light, and Energy

**8.3.1. Introduction to Light.** Molecules are far too small for us to see, and the motions of their atoms are far too fast for us to observe directly. To actually understand what happens to coenzyme B<sub>12</sub> when bound to EAL, we need something to act as a probe, something that will interact with B<sub>12</sub> and produce some sort of effect that we can measure. Thankfully, there is a whole field of science on the interface of chemistry and physics called **spectroscopy**, the study of the interaction of light and matter. We can literally shine light on our problem, and use it to interrogate our molecules. The experiments I will describe to you here are types of **absorption** spectroscopy, in which the amount of light absorbed by a sample is measured. Unless you are colorblind, your eyes perform experiments like this all the time. For example, look at the vial in Figure 8.3 below, which contains a dilute solution of coenzyme B<sub>12</sub>. The lights in the room are shining white light on it, which consists of all colors of light. Some of these colors are absorbed by the solution, while others are transmitted. Your eye detects the latter, and interprets that combination of colors as "red."

It is possible to make this measurement of color more quantitative, but to do so we'll first have to talk about what light is. The technical term for light is **electromagnetic radiation**, a fancy phrase that tells us that light behaves a lot like waves in the ocean or ripples in a pond. For example,



if you drop a rock into a pond, you will see ripples “radiating” away from the point where the rock hits the water. If we want to describe one of these ripples, we might talk about its **amplitude** (how high and low the water’s crests and troughs are), **wavelength** (the distance between one crest and another), or **frequency** (how many times the wave goes up and down in a certain amount of time). We use the same terms to describe light waves, which are oscillations of an electric field.<sup>7</sup> In the early 20<sup>th</sup> century, Einstein made the revolutionary suggestion that the energy of light is proportional to its frequency and inversely proportional to its wavelength. Put more simply, high-energy light has a high frequency and a short wavelength, while low-energy light has a low frequency and a long wavelength. When your eye distinguishes different colors of light, it is really identifying what wavelengths of light are reaching it. Red light has the longest wavelength of light you can detect, and violet light has the shortest.<sup>8</sup>



**Figure 8.3.** A dilute solution of coenzyme B<sub>12</sub>.

So why does the sample in Figure 8.3 absorb some colors of light and not others? The best answers we have come from the field of quantum mechanics. One of the most important results that follows from the fundamental postulates of quantum mechanics is that electrons can only exist

---

<sup>7</sup> Technically, there are two fields oscillating: one electric and one magnetic. But to keep this chapter from growing too long, we’ll just talk about the electric field.

<sup>8</sup> There are wavelengths of light much longer and shorter than these. While they’re important for other interesting types of spectroscopy, the experiments discussed in this chapter use primarily visible light.

in certain configurations called **states**, and that each state has a characteristic energy associated with it.<sup>9</sup> Ordinarily, the electrons in atoms and molecules are in their lowest-energy state (called the **ground state**), but if something provides them with energy, an electron can be moved into a higher-energy **excited state**. Under no circumstances, however, should an electron have any amount of energy that doesn't correspond to one of its possible states.

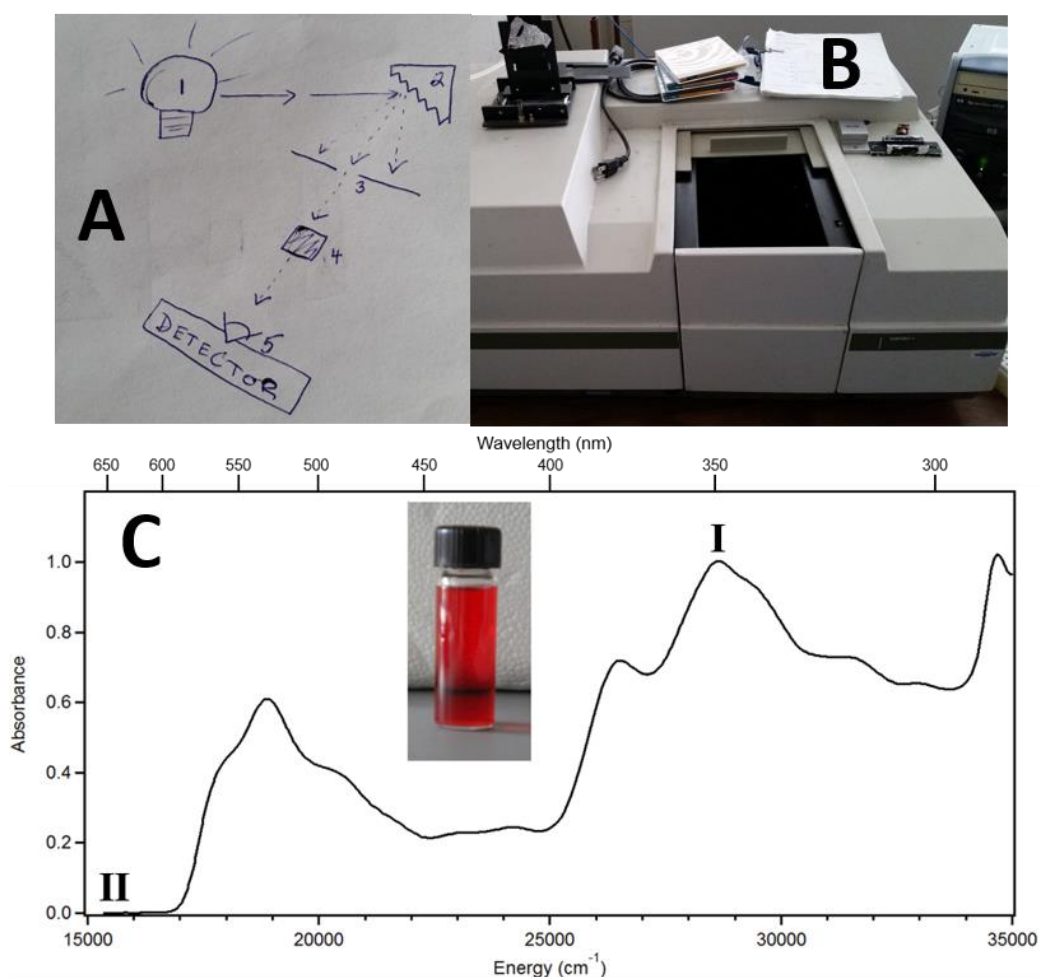
If that last paragraph was a doozy, just know that it was meant to provide a short introduction to this statement: **electrons are only allowed to have certain energies**. To get from a ground state to an excited state, they need something to provide that extra energy, which is what happens when light is absorbed. Light that has energy equal to the difference between an electron's ground and excited states **may** be absorbed by the electron to move it into an excited state. But if the light that shines on a sample does not have the right amount of energy, it won't be absorbed and instead will pass through. Since different colors of light have different energies, this gives a general explanation for why solutions like the one in Figure 8.3 have color – red light just doesn't have the right energy to be absorbed.

**8.3.2 Spectroscopic and Computational Techniques.** Since we are now equipped with some knowledge about how light and matter interact, we can talk about how to design an experiment to better understand why the solution appears a certain color. Figure 8.4A below shows a simplified sketch of a **spectrophotometer**, the instrument we use to collect this type of data. White light is emitted from a source (1) and is reflected off a “diffraction grating” (2) which, like a prism, separates the different wavelengths (colors). Only light from a very narrow range of colors is able to pass through a slit (3) and make it to our sample (4). Some of that light will be absorbed

---

<sup>9</sup> If that doesn't make sense at first, try repeating it a few times. It may never sound reasonable, but eventually you may convince yourself that it's true.

by the sample, and the rest will make it to a detector (5). The detector measures the intensity of this light relative to the intensity at the source, and uses that to calculate the **absorbance**, a measure of how much light is absorbed by the sample. After the absorbance has been measured at one wavelength, the angle of the diffraction grating is changed to allow a different wavelength of light through the slit. The actual instrument we use to perform this measurement is shown in Figure 8.4B.



**Figure 8.4.** (A) Sketch of how an absorption spectrophotometer works. (B) A picture of the spectrophotometer in our lab. (C) An absorption spectrum of coenzyme B<sub>12</sub> produced by this instrument.

The end result of this experiment is an absorption spectrum (Figure 8.4C). This plot has absorbance on the y-axis plotted versus wavelength, frequency, or energy on the x-axis.<sup>10</sup> If the incoming light has the appropriate amount of energy to move any of a molecule's electrons into a higher-energy state, the molecule **may** absorb that energy, in which case there will be a correspondingly high absorbance. For example, light with a wavelength of 350 nm (energy  $\approx 28,500 \text{ cm}^{-1}$ ) is very easily absorbed by coenzyme B<sub>12</sub>, so at that point (I) on our plot we have a high absorbance and a "peak" in the spectrum. However, light at 650 nm (point II, energy  $\approx 15,400 \text{ cm}^{-1}$ ) does not have the right amount of energy to excite an electron, and so it is not absorbed at all.

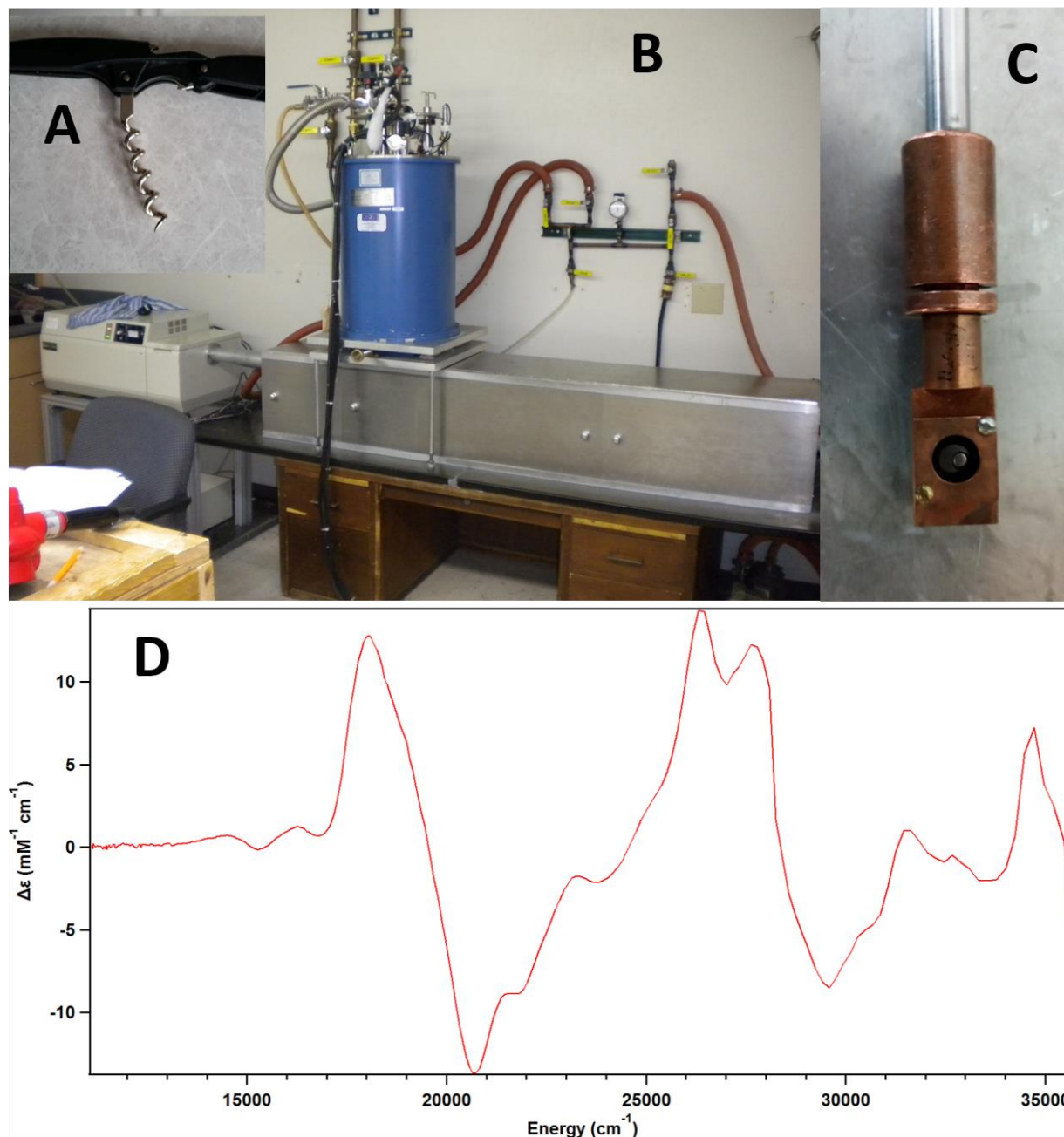
There is clearly a lot of information about coenzyme B<sub>12</sub> in this figure, but there are also two major obstacles preventing us from obtaining and using that information. First, even though we can find the peaks on the spectrum, we don't know for sure how many possible transitions there are. That's because each peak has some width to it. It's certainly possible that each peak is due to one and only one transition, but it's also possible that there are multiple transitions very close in energy that overlap with one another. In other words, our absorption spectrum suffers from poor resolution. Second, even if we knew how many transitions we had and what the corresponding energies were, we don't know how to interpret that information. We may know that the energy is a difference between two states an electron may be in, but the spectrum itself can't tell us where the electron is coming from and where it is going to for any individual transition.

To resolve our first issue, we use a complementary technique called magnetic circular dichroism (MCD) spectroscopy, which requires us to do two things. First, we'll conduct our

---

<sup>10</sup> In our group, we usually use energy, in the unusual unit of "wavenumbers" ( $\text{cm}^{-1}$ ). In Figure 0.4, I also included wavelength in "nanometers" (nm) as an upper x-axis.

experiment inside a high magnetic field. Next, instead of ordinary light, we use “circularly polarized” light, in which the electric field looks more like a corkscrew than a wave (Figure 8.5A). This light can be either “right” or “left” circularly polarized light, depending on whether it is turning in a clockwise or counterclockwise direction. The rest of the experiment follows the same principles as absorption spectroscopy, which we talked about earlier, but the setup is much more challenging. Our instrument is shown in Figure 8.5B: circularly polarized light is generated by the beige box on the far left and passes through the metal tube to a sample chamber beneath the blue cylinder. That cylinder is a magnetocryostat, which contains a superconducting magnet and a liquid helium cryostat to keep it and our samples very, very cold. The sample itself is injected into a small cell and screwed onto a rod (Figure 8.5C), which is lowered through the magnetocryostat and into the sample chamber. After the light passes through the sample, it goes all the way to a detector at the far end of the gray box. Unlike an absorption spectrum, which can be collected in a few minutes, it takes hours to collect MCD spectra, and the instrument requires frequent maintenance while it runs.



**Figure 8.5.** Setup and results of an MCD experiment. (A) Circularly polarized light has an electric field that oscillates and rotates, which makes it look like it's traveling along a corkscrew. An observer watching the light come down this corkscrew toward her would see it rotating counterclockwise, so this would be "left" circularly polarized light. (B) The MCD instrument used to collect data in this chapter. (C) Close-up of the sample cell and rod. (D) An MCD spectrum of coenzyme B<sub>12</sub>.

So why do we go to all this trouble? You may have noticed that the features of the absorption spectrum in Figure 8.4C have different intensities. This is a result of what chemists call “selection rules”, rules that determine how likely a transition will be based on the initial and final states of the electron.<sup>11</sup> Interestingly, these selection rules can change when we impose certain conditions on our experiment – conditions like the use of circularly polarized light or an external magnetic field. That means that prominent features in the absorption spectrum may be much less intense in an MCD spectrum, and vice versa. We can also play a clever trick by using both right- and left-circularly polarized light, since our molecules will sometimes absorb one better than the other. That’s a huge aid to our problem of resolution, since we can take the difference in absorbance between the two polarizations of light and get a spectrum with both positive and negative features, as shown in the MCD spectrum in Figure 8.5D. Two transitions that might overlap in the absorption spectrum will be easily distinguished in the MCD spectrum if one is positive and the other is negative. Again, the same transitions are being probed in both absorption and MCD spectroscopy; by using both simultaneously we have a better chance of finding all of the transitions and precisely determining their energies.

Overcoming our second obstacle, the one of interpretation, requires another complementary technique, but this one doesn’t involve going into the lab. Instead, we attempt to use the theories of quantum mechanics to predict the energies at which we should observe transitions, and how intense those transitions will be. Unfortunately, the equations involved can’t be solved exactly for atoms or molecules with more than one electron – and we have tens of thousands of them! In the past few decades, however, a number of very good approximate methods

---

<sup>11</sup> I’m being deliberately vague here in order to save you another mini-lecture on quantum mechanics.

have been developed that can be applied to systems of our size. These methods allow us to predict the **geometric structure** (the relative positions of atoms and their nuclei) and **electronic structure** (the arrangement and energies of electrons) of EAL, coenzyme B<sub>12</sub>, and ethanolamine. Despite the fact that these methods aren't exact, it can take a large computer cluster weeks to finish computing the final position of all the atoms of just one of my models, after which it takes about a week for a separate calculation of the absorption spectrum to run.

These computations are worth all this time because of the huge wealth of easily interpretable information they contain. When the computer predicts that an electron in B<sub>12</sub> will absorb light of a certain energy, it can also tell me where the electron is coming from and where it is going to. This information gives me clues about what EAL does to B<sub>12</sub> to affect the electron's energies. We can also use our computational model to correlate changes in the computed spectrum to changes in the molecule's geometry. For example, if we see that binding to EAL causes one of the bonds in B<sub>12</sub> to get longer, we can investigate whether or not that change is responsible for a corresponding change in the computed spectra. In the computer models, I can manipulate that bond length to any arbitrary distance and see the effect on the absorption spectrum, which is something I could never do to my real molecules in the lab.

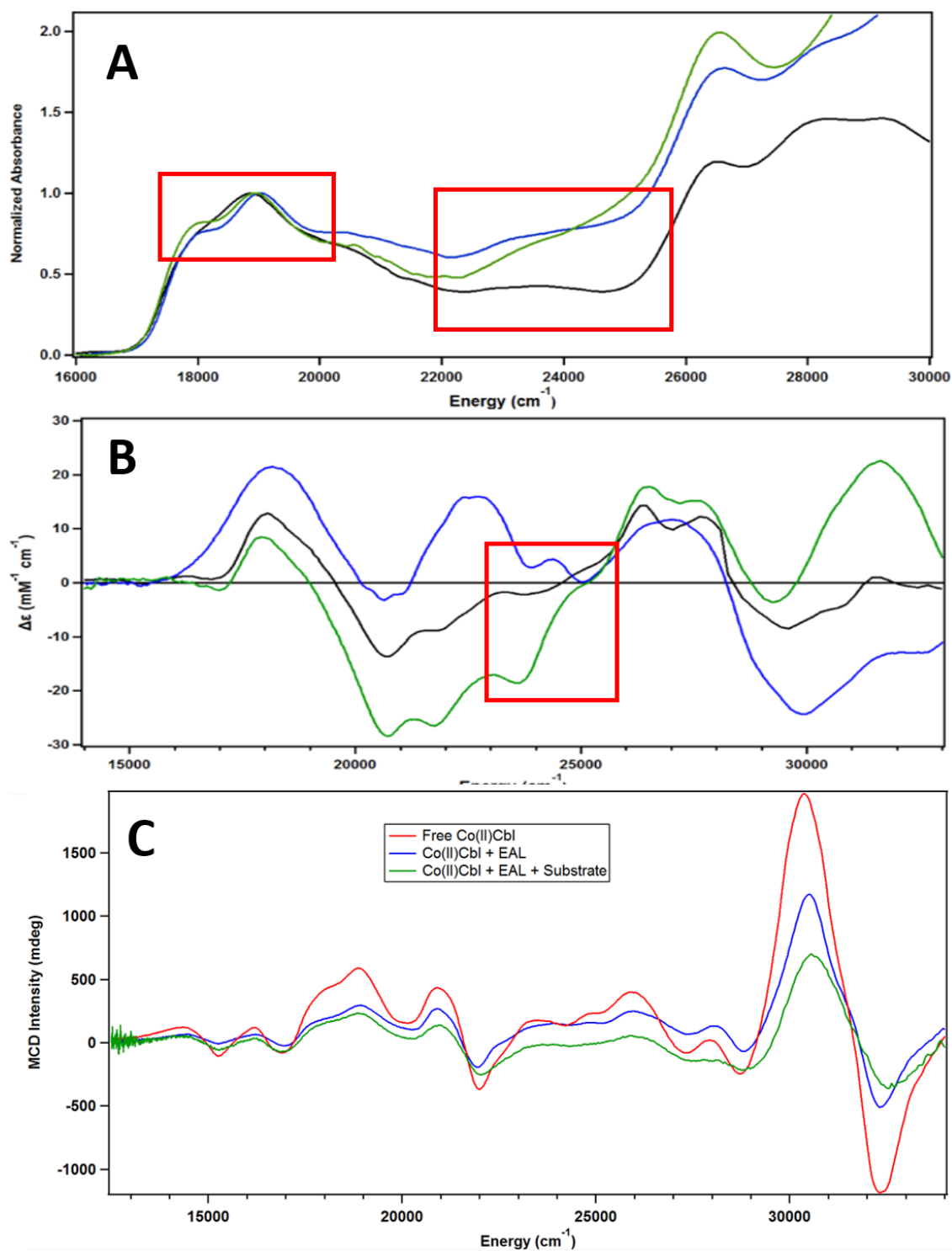
The wealth of the information we get should be taken with a grain of salt, however, since these methods are approximate. Every time we use one of these models to explain something about B<sub>12</sub>'s behavior, we must be sure that the theory has been experimentally verified. That is, we calculate something with that theory that can be compared to our experimental data. If experiment and theory agree, then it's evidence that the theory can be trusted. This shouldn't be mistaken for ironclad proof that our theories are perfect: on the fringes of science, we seek adequacy, not perfection. To further demonstrate that the theory is good enough, we can use the computational



results to propose hypotheses about how B<sub>12</sub> functions, and then design further experiments to test those hypotheses. When everything works right, this approach is circular, since good computations will inspire new experiments, which will in turn require further computations to interpret.

## 8.4 Results

**8.4.1. A mechanism for EAL: to stabilize or destabilize?** Now that you've been introduced to the systems I study and the techniques I use, let's get to the data. As I suggested earlier, the first thing we want to determine is how EAL affects the Co–C bond. In principle, it could do this by either weakening the bond directly or by stabilizing Co(II)Cbl to make it easier to reach that state. We can determine this by comparing the absorption and MCD spectra of coenzyme B<sub>12</sub> before and after we bind it to EAL. We can also do the same to Co(II)Cbl. Those are the comparisons shown in Figure 8.6 below. The top two overlays show the absorption and MCD spectra of coenzyme B<sub>12</sub> by itself, with EAL, and with EAL and ethanolamine. The bottom part of the figure shows the corresponding MCD spectra of Co(II)Cbl. It's possible to talk about the gritty details about this spectra – and I do in Chapter 3 – but to get the main point, we only need to look at these spectra very qualitatively. Here's what I mean: when EAL and ethanolamine are added to coenzyme B<sub>12</sub>, there are some pretty noticeable changes (see the red boxes in particular). We'll talk about what causes these in Section 8.4.2, but for now, note that there are almost no changes to the MCD spectrum of Co(II)Cbl when we do the same to it.



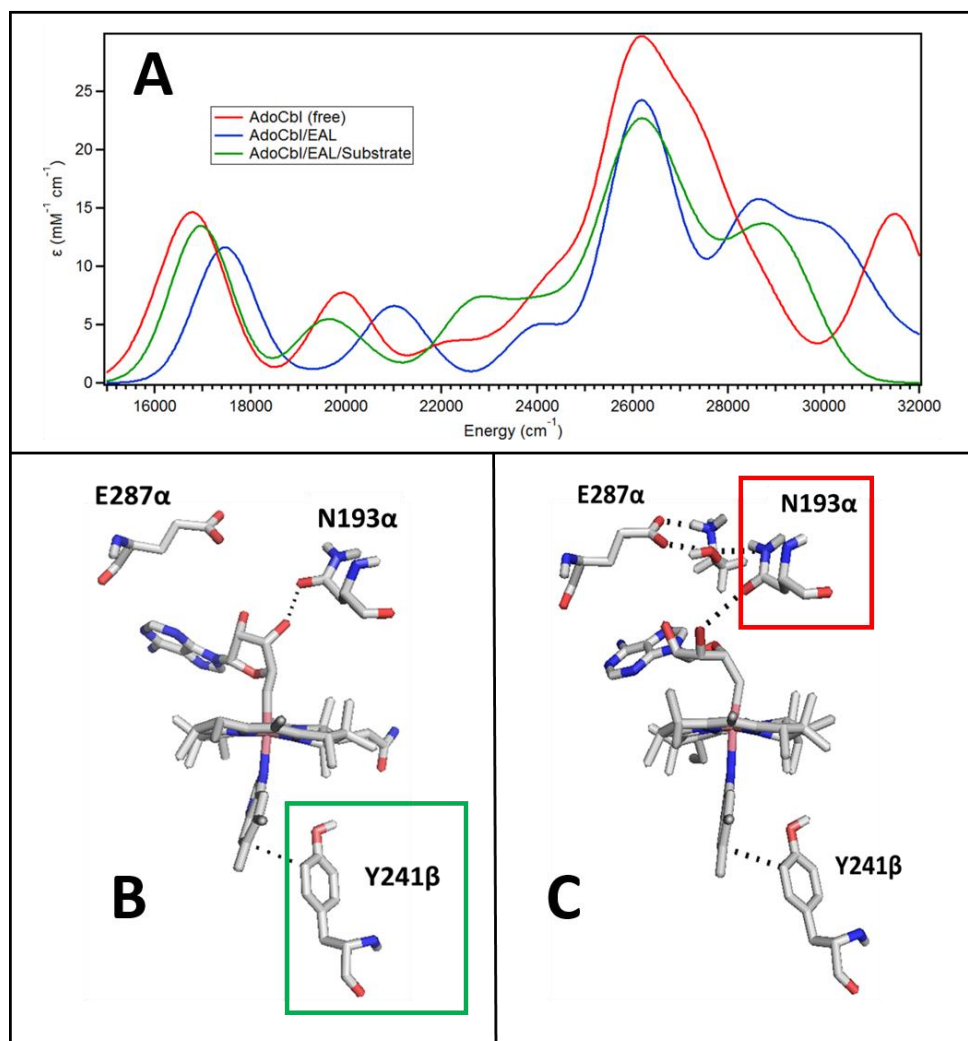
**Figure 8.6.** Absorption (A) and MCD (B) spectra of coenzyme B<sub>12</sub> and MCD spectra of Co(II)Cbl (C) in the following three states: by itself (red), with the addition of EAL (blue), and with the addition of EAL and ethanolamine (green). Key changes induced by the protein and substrate are indicated by red boxes.

That's really important, because it tells us that EAL must function by weakening the Co–C bond directly. What's interesting is that this is the exact opposite of what we would observe if we did this experiment with the histidine-binding class of coenzyme B<sub>12</sub>-dependent enzymes. In those enzymes, nothing happens to the spectra of the coenzyme B<sub>12</sub> form, but a lot happens to the Co(II)Cbl form. This strongly suggests that for some reason, whether or not the enzyme uses a histidine side chain to bind our cofactor is related to whether the enzyme works by destabilizing coenzyme B<sub>12</sub> or by stabilizing Co(II)Cbl. That alone is a worthwhile thing to know, but we can extend our analysis to find out what parts of EAL are important and why.

**8.4.2. Proposed roles for amino acids.** The results from the previous experiment can only give us information about the total effect of the protein on the cofactor. To dig deeper, we need to use a computational model. I'll spare you the details of the model and show you the results of the absorption spectra I compute in Figure 8.7A. Compare that figure to the spectra to the coenzyme B<sub>12</sub> absorption spectra in Figure 8.6A above, and judge for yourself whether or not the model reproduces the major effects of EAL and ethanolamine on the cofactor's spectrum. I hope you'll agree with me that theory and experiment are in good agreement here.<sup>12</sup> We can now use the results of our computations to propose reasons for why the spectra are changing.

---

<sup>12</sup> If not, then in principle you shouldn't accept the model I'm about to present to you – but thank you for reading this far!



**Figure 8.7.** Results from my computational models. (A) Absorption spectra for coenzyme B<sub>12</sub> in three states: by itself (red), with the addition of EAL (blue), and with the addition of EAL and ethanolamine. (B) Geometric structures of coenzyme B<sub>12</sub> bound to EAL. (C) Geometric structure of coenzyme B<sub>12</sub> bound to EAL in the presence of ethanolamine.

Figures 8.7B and 8.7C show the molecular geometry of coenzyme B<sub>12</sub> used to compute the blue and green spectra in Figure 8.7A. (They also include three side chains of EAL's amino acids, identified by an alphanumeric code.) Since these models have been validated, we can ask ourselves: what parts of the protein are causing our cofactor's shape to change? And that brings us to the final part of the study. It turns out that when we only include B<sub>12</sub> and EAL (Figure 8.7B), the major changes all involve the lower half of the cofactor. And it appears that the amino acid

responsible for that movement is the one in the green box. That's the side chain of tyrosine – a big, bulky amino acid – that looks to be imposing on the DMB of B<sub>12</sub>. When we add ethanolamine to the mix (Figure 8.7C) we see all the changes happening to the upper half of the cofactor. Here, the culprit is the one in the red box, another big amino acid called asparagine. Asparagine has both a strong attraction to the ethanolamine molecule and to the cofactor, and in order to stay close to both, it needs to distort the cofactor's geometry, causing the Co–C bond to grow longer. In general, longer bonds are weaker bonds, so this is a tell-tale sign of coenzyme B<sub>12</sub> being destabilized in the presence of EAL and ethanolamine. Thus, the specific changes caused by these tyrosine and asparagine side chains in the model's prediction agree with the general mode of coenzyme B<sub>12</sub> destabilization that we qualitatively expect based on our experiments.

## 8.5 Conclusion

An important step in the scientific method is the formulation of theories that tie together a series of observations. In the previous section, I showed you the following:

1. The absorption and MCD spectra of coenzyme B<sub>12</sub> are changed by EAL and ethanolamine, meaning they affect the cofactor's electronic structure.
2. The absorption and MCD spectra of Co(II)Cbl are not affected by EAL and ethanolamine.
3. Our computer models are verified by reproducing the changes to the spectra observed experimentally in #1.
4. These computer models predict changes to the geometric structure of coenzyme B<sub>12</sub> when EAL and ethanolamine are added.
5. The structural changes in #4 appear to be a result of the effects of two specific amino acids that are near the cofactor.

I would like to tie these observations together in a conclusion by using my favorite analogy, one that makes it into my informal presentations but not (usually) into my papers: **coenzyme B<sub>12</sub> is like a bottle of beer, and EAL and ethanolamine together act as a bottle opener.** In this analogy, the goal of EAL and ethanolamine is to take the top off of coenzyme B<sub>12</sub>'s "bottle" by breaking the Co–C bond. This happens in two steps. First, before ethanolamine arrives, EAL binds the cofactor in a very rigidly defined position, just as you might firmly grasp a bottle that you plan to open. This is one possible role of the tyrosine side chain we identified: by imposing a certain position on the DMB group, it prevents the cofactor from wiggling around inside the enzyme. Then, when ethanolamine arrives, it provides a motive for the asparagine side chain to drag across the cofactor's upper face and pull on the Co–C bond, thus weakening it. Once the bond is broken, the strain that was placed on the cofactor is removed, explaining why we see no evidence of major changes to the Co(II)Cbl spectra.

That is the conclusion of this study, and although I hope it has helped you get a glimpse of my research, I also hope that it leaves you hanging a little bit. What I've told you about should be seen not as a complete work, but as just one rung of a ladder. It is only possible because of the countless hours of work put in by biologists, biochemists, chemists, and physicists, not to mention the funding support provided by my university, the taxpayers that fund it, and the federal agencies that deemed it worthy of pursuit. And it is clearly not the culmination of our work on B<sub>12</sub>. Like any conclusion on the fringes of our scientific knowledge, mine are supported by evidence and yet to some extent speculative. That's a good thing! My hypothesis regarding these tyrosine and asparagine side chains is that both are necessary for EAL to function, and I hope that some young researcher comes along and designs new experiments to test this. This is how scientific progress

tends to happen, with every small project giving us a little more understanding of how the world works, and a lot more questions to answer.